

Figure #1

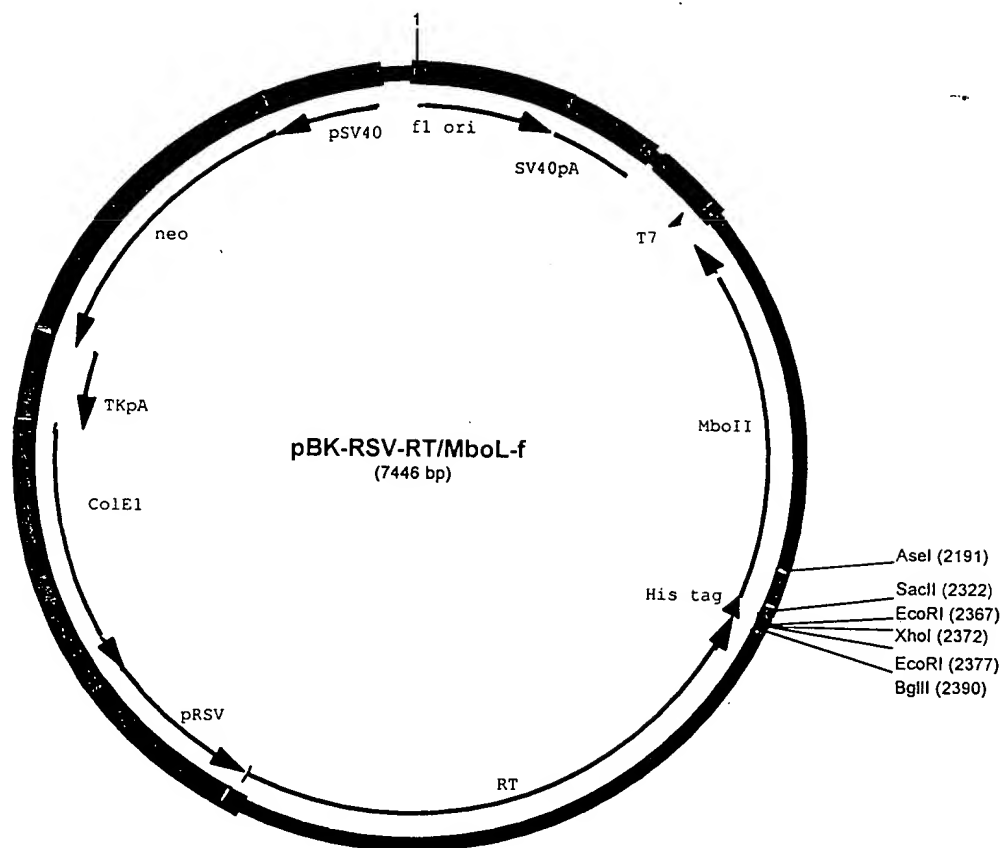


Figure 1: Structure of the pssDNA-Express A plasmid. The reverse transcriptase and *Mbo* II genes are expressed in a single polypeptide for which the message is transcribed from the RSV promoter. The reverse transcriptase and *Mbo* II domains are separated by a histidine- and proline-rich linker.

Figure #2

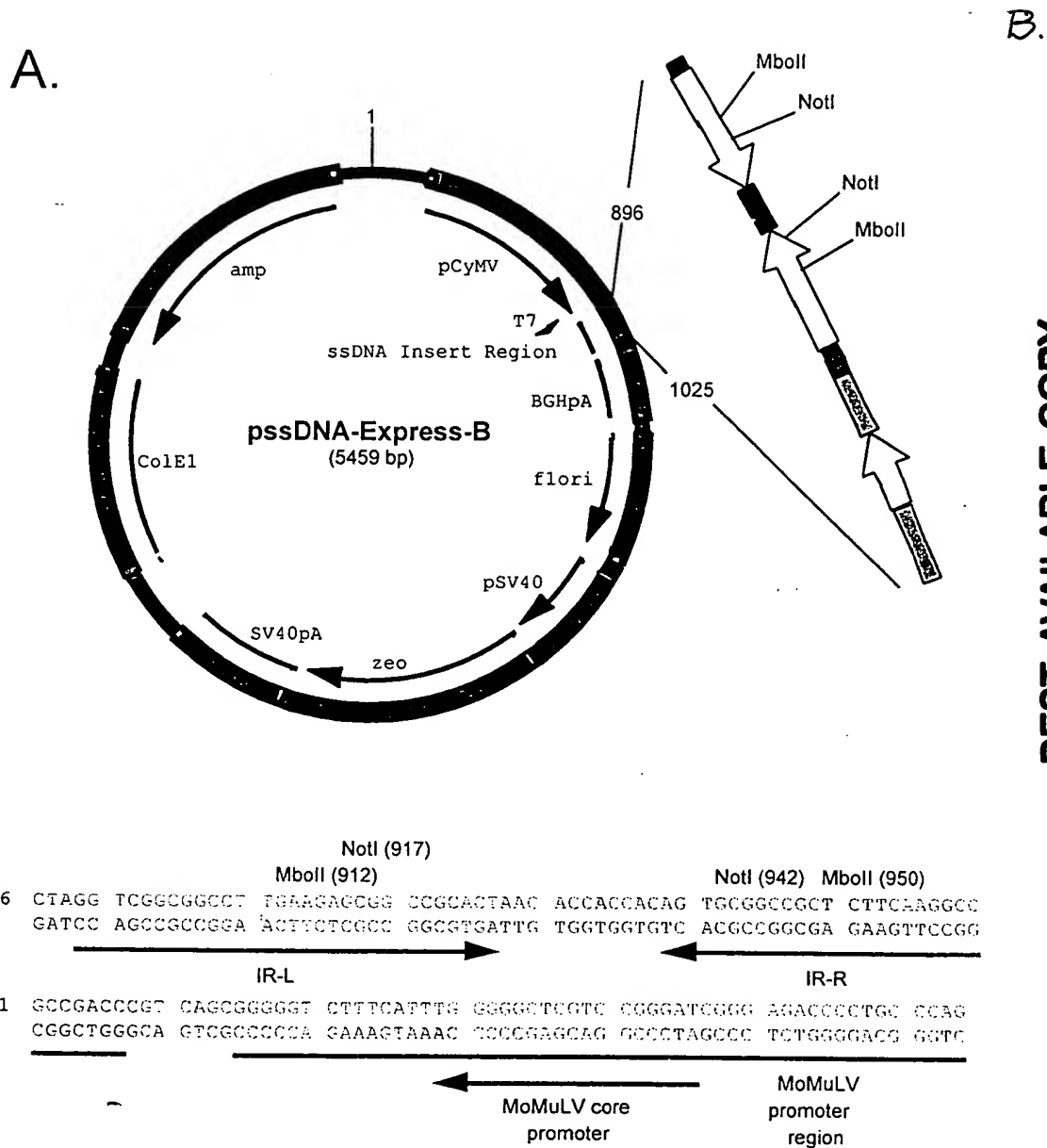


Figure 2: Structure of the pssDNA-Express-B plasmid. A. Plasmid map with enlargement showing arrangement of insert features: inverted complementary repeats (open arrows); MoMuLV promoter region (hatched) and MoMuLV core promoter (stippled arrow). B. Sequence of the insert region.

BEST AVAILABLE COPY

1990-1991
 1992-1993
 1994-1995
 1996-1997
 1998-1999
 2000-2001
 2002-2003
 2004-2005
 2006-2007
 2008-2009
 2010-2011
 2012-2013
 2014-2015
 2016-2017
 2018-2019
 2020-2021
 2022-2023
 2024-2025
 2026-2027
 2028-2029
 2030-2031
 2032-2033
 2034-2035
 2036-2037
 2038-2039
 2040-2041
 2042-2043
 2044-2045
 2046-2047
 2048-2049
 2050-2051
 2052-2053
 2054-2055
 2056-2057
 2058-2059
 2060-2061
 2062-2063
 2064-2065
 2066-2067
 2068-2069
 2070-2071
 2072-2073
 2074-2075
 2076-2077
 2078-2079
 2080-2081
 2082-2083
 2084-2085
 2086-2087
 2088-2089
 2090-2091
 2092-2093
 2094-2095
 2096-2097
 2098-2099
 2100-2101
 2102-2103
 2104-2105
 2106-2107
 2108-2109
 2110-2111
 2112-2113
 2114-2115
 2116-2117
 2118-2119
 2120-2121
 2122-2123
 2124-2125
 2126-2127
 2128-2129
 2130-2131
 2132-2133
 2134-2135
 2136-2137
 2138-2139
 2140-2141
 2142-2143
 2144-2145
 2146-2147
 2148-2149
 2150-2151
 2152-2153
 2154-2155
 2156-2157
 2158-2159
 2160-2161
 2162-2163
 2164-2165
 2166-2167
 2168-2169
 2170-2171
 2172-2173
 2174-2175
 2176-2177
 2178-2179
 2180-2181
 2182-2183
 2184-2185
 2186-2187
 2188-2189
 2190-2191
 2192-2193
 2194-2195
 2196-2197
 2198-2199
 2200-2201
 2202-2203
 2204-2205
 2206-2207
 2208-2209
 2210-2211
 2212-2213
 2214-2215
 2216-2217
 2218-2219
 2220-2221
 2222-2223
 2224-2225
 2226-2227
 2228-2229
 2230-2231
 2232-2233
 2234-2235
 2236-2237
 2238-2239
 2240-2241
 2242-2243
 2244-2245
 2246-2247
 2248-2249
 2250-2251
 2252-2253
 2254-2255
 2256-2257
 2258-2259
 2260-2261
 2262-2263
 2264-2265
 2266-2267
 2268-2269
 2270-2271
 2272-2273
 2274-2275
 2276-2277
 2278-2279
 2280-2281
 2282-2283
 2284-2285
 2286-2287
 2288-2289
 2290-2291
 2292-2293
 2294-2295
 2296-2297
 2298-2299
 2300-2301
 2302-2303
 2304-2305
 2306-2307
 2308-2309
 2310-2311
 2312-2313
 2314-2315
 2316-2317
 2318-2319
 2320-2321
 2322-2323
 2324-2325
 2326-2327
 2328-2329
 2330-2331
 2332-2333
 2334-2335
 2336-2337
 2338-2339
 2340-2341
 2342-2343
 2344-2345
 2346-2347
 2348-2349
 2350-2351
 2352-2353
 2354-2355
 2356-2357
 2358-2359
 2360-2361
 2362-2363
 2364-2365
 2366-2367
 2368-2369
 2370-2371
 2372-2373
 2374-2375
 2376-2377
 2378-2379
 2380-2381
 2382-2383
 2384-2385
 2386-2387
 2388-2389
 2390-2391
 2392-2393
 2394-2395
 2396-2397
 2398-2399
 2400-2401
 2402-2403
 2404-2405
 2406-2407
 2408-2409
 2410-2411
 2412-2413
 2414-2415
 2416-2417
 2418-2419
 2420-2421
 2422-2423
 2424-2425
 2426-2427
 2428-2429
 2430-2431
 2432-2433
 2434-2435
 2436-2437
 2438-2439
 2440-2441
 2442-2443
 2444-2445
 2446-2447
 2448-2449
 2450-2451
 2452-2453
 2454-2455
 2456-2457
 2458-2459
 2460-2461
 2462-2463
 2464-2465
 2466-2467
 2468-2469
 2470-2471
 2472-2473
 2474-2475
 2476-2477
 2478-2479
 2480-2481
 2482-2483
 2484-2485
 2486-2487
 2488-2489
 2490-2491
 2492-2493
 2494-2495
 2496-2497
 2498-2499
 2500-2501
 2502-2503
 2504-2505
 2506-2507
 2508-2509
 2510-2511
 2512-2513
 2514-2515
 2516-2517
 2518-2519
 2520-2521
 2522-2523
 2524-2525
 2526-2527
 2528-2529
 2530-2531
 2532-2533
 2534-2535
 2536-2537
 2538-2539
 2540-2541
 2542-2543
 2544-2545
 2546-2547
 2548-2549
 2550-2551
 2552-2553
 2554-2555
 2556-2557
 2558-2559
 2560-2561
 2562-2563
 2564-2565
 2566-2567
 2568-2569
 2570-2571
 2572-2573
 257

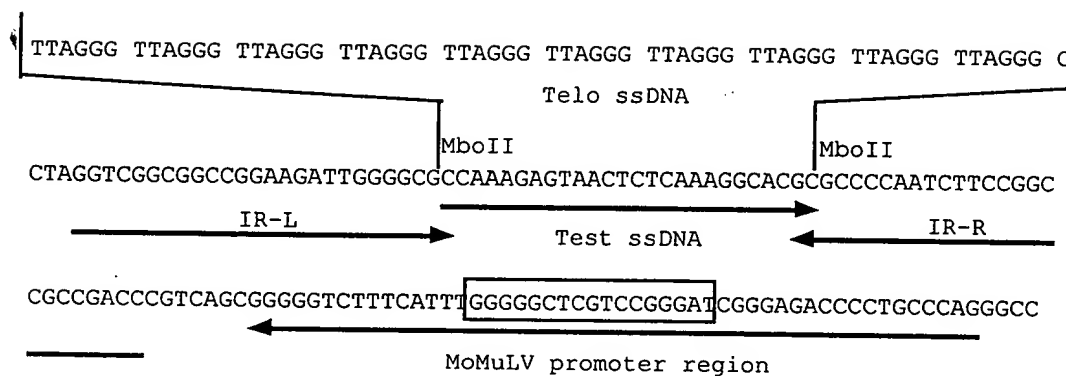


Figure #4

JUNE 21, 1997

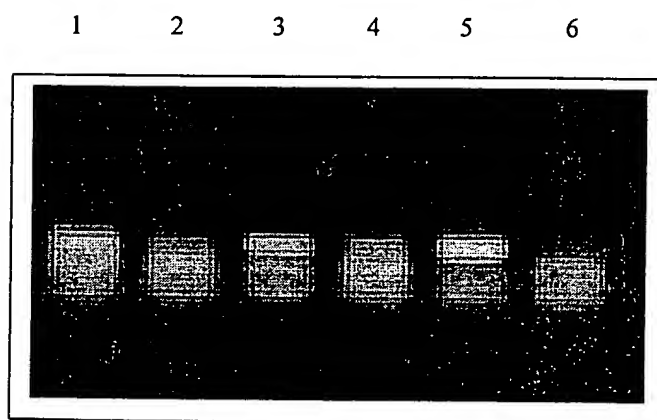
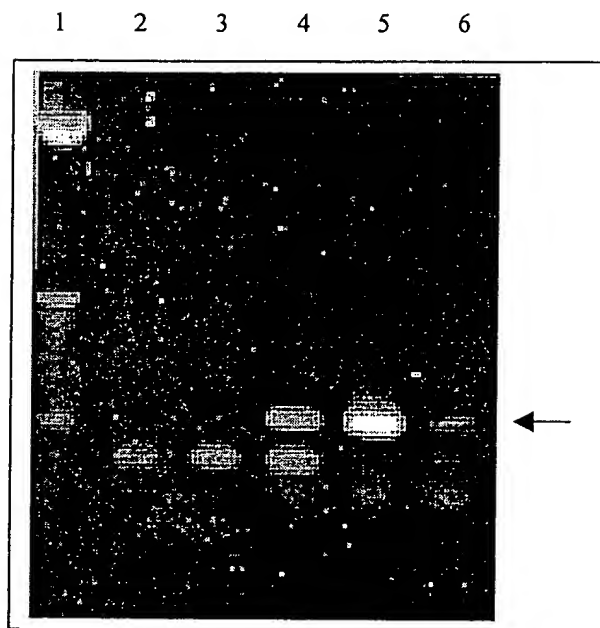


Figure 4: Reverse transcriptase assays on RNA/ssDNA extracts from transformed HeLa cell lines. A. Transient transformations. Lanes: (1) size markers; (2) untransformed HeLa cells; (3), HeLa cells transformed with the vector pcDNA3.1Zeo⁺; (4) HeLa cells transformed with pssXA; (5) positive control containing 2 milliunits of MoMuLV reverse transcriptase; (6) negative control containing no protein extract. The arrow indicates the 150 bp amplification product. (Bk2p106)

B. Reverse transcriptase assays on stably transformed cell lines. Lanes: (1) clone A12, (2) B8, (3) B12, (4) D7, (5) positive control containing 2 mU of MoMuLV reverse transcriptase, (6) Control containing no extract. (Bk2p152)

BEST AVAILABLE COPY

Figure #5

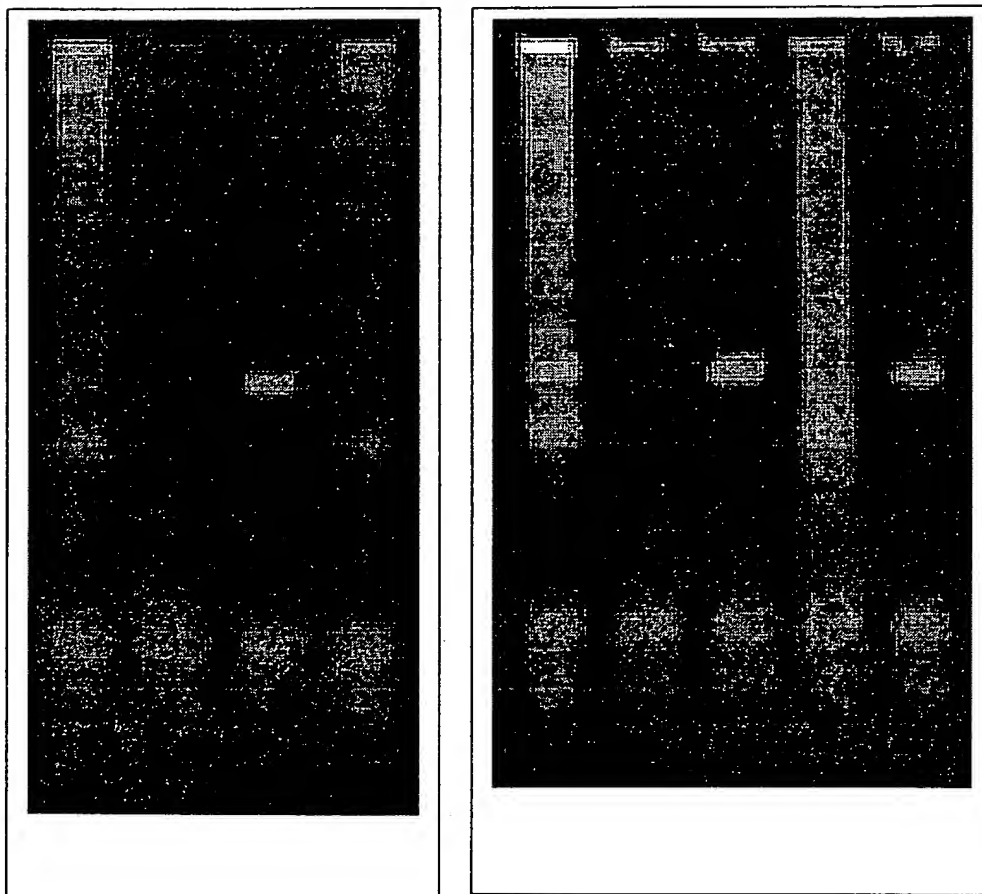


Figure 5: Amplification of single-stranded DNA from extracts of transformed cells. RNA/ssDNA preparations were used as templates in PCR reactions using primers specific to the expected ssDNA product. Left panel, lanes 1-3: total RNA/ssDNA PCR template isolated from stably transformed colony A12 (1) used with no prior treatment (2) treated with S1 nuclease, (3) treated with RNase A. Lane (4) total RNA/ssDNA from a colony stably transformed with the pcDNA3.1Zeo+ vector alone. (Bk3p12a lanes5-8) Right panel, lanes 1-3,; total RNA/ssDNA PCR template isolated from stably transformed colony B12 (1) used with no prior treatment (2) treated with S1 nuclease, (3) treated with RNase A. Lane (4), total RNA/ssDNA from a colony stably transformed with the pcDNA3.1Zeo+ vector alone. Lane (5), positive control template, plasmid pTest.

BEST AVAILABLE COPY

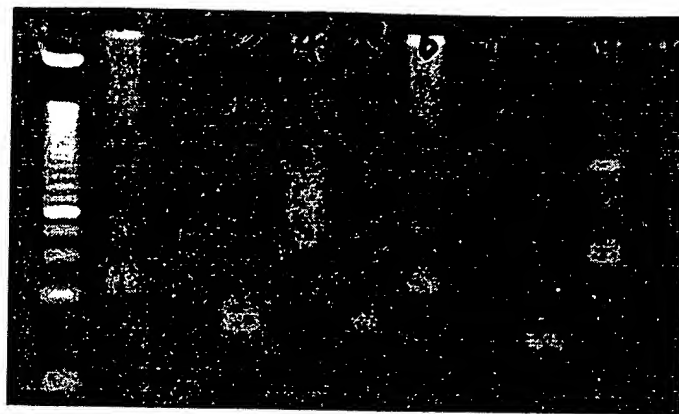
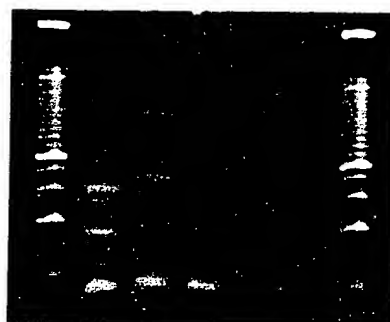


FIG. 6

1. 2. 3. 4. 5. 6. 7.



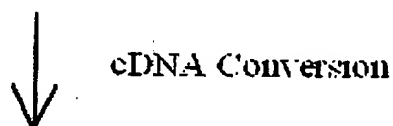
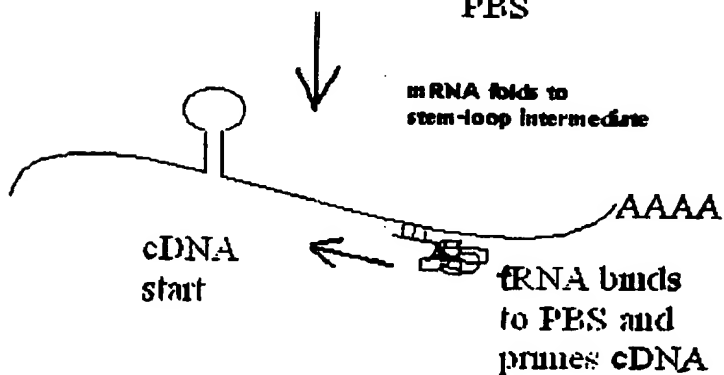
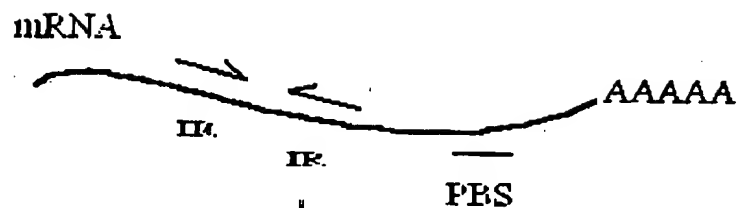
Extension loop-
Stem juncture-

25 b.p.
ladder

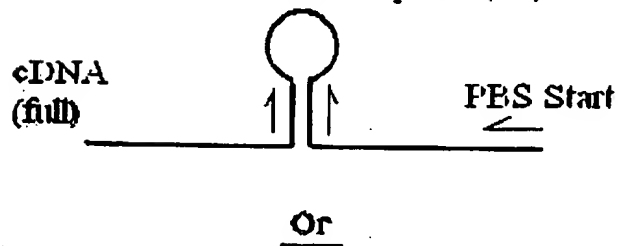
FIG. 7

003782-00450

BEST AVAILABLE COPY



Stem-loop Structure
from Inverted Repeats (IR)



cDNA Pre-mature
Termination from Stem-loop



BEST AVAILABLE COPY